

In the claims:

Please amend the claims as follows:

1. (Currently Amended) A method for mutagenesis comprising:
annealing one or more primers having a nucleotide sequence containing at least one mutation and a phosphorylated 5'-terminus, to a DNA template;
elongating the annealed primer or primers by using a DNA polymerase;
ligating the phosphorylated 5'-terminus and the elongated terminus of the primer or primers by means of a DNA ligase to synthesize a circular DNA containing said primer or primers;
denaturing the circular DNA;
repeating the reactions of annealing, elongating, ligating, and denaturing to amplify the circular DNA to generate DNA products including a multiple copies of a single-stranded circular DNA containing the primer or primers;
selectively digesting the DNA products other than the single-stranded circular DNA to produce megaprimer fragments;
annealing said megaprimer fragments to said single-stranded circular DNA; and
elongating the annealed megaprimer fragments by using said DNA polymerase to synthesize a double stranded DNA.
2. (Previously Amended) The method for mutagenesis according to Claim 1 wherein said primers are used to introduce mutations at multiple sites simultaneously.
3. (Previously Amended) The method for mutagenesis according to Claim 1, wherein said primers comprise degenerative primers to introduce random mutations at certain sites in a nucleotide sequence.
4. (Previously Amended) The method for mutagenesis according to Claim 1, further comprising:

before annealing the megaprimer fragments, adding an auxiliary primer complementary to a region adjacent to the nucleotide sequence in which mutations are introduced.

5. (Previously Amended) The method for mutagenesis according to Claim 4 wherein said auxiliary primer is a T7 primer.

6. (Previously Amended) The method for mutagenesis according to Claim 1 further comprising:
digesting selectively the other DNA products by methylated and hemi- methylated nucleotide sequences are selectively cut.

7. (Previously Amended) The method for mutagenesis according to Claim 1 wherein DpnI is used to selectively digest the DNA products.

8. (Previously Amended) The method for mutagenesis according to Claim 1 wherein in elongating the primer or primers, a thermostable high-fidelity DNA polymerase is used, and in ligating the phosphorylated 5'-terminus and the elongated terminus, a thermostable DNA ligase is used.

9. (Previously Amended) The method for mutagenesis according to Claim 8, wherein the method is conducted in a reaction solution comprising at least said primers, said template DNA, said thermostable high-fidelity DNA polymerase and said thermostable DNA ligase.--

10. (Currently Amended) The method of mutagenesis according to claim 1 wherein the DNA products further comprises: a single-stranded circular DNA without the primer or primers, a single-stranded circular DNA with the primer or primers annealed to the DNA template to form a double-stranded circular DNA, and a single-stranded circular DNA without the primer or primers annealed to the DNA template to form another double-stranded circular DNA.

11. (Currently Amended) A method for mutagenesis comprising:

annealing one or more primers having a nucleotide sequence containing at least one mutation and a phosphorylated 5'-terminus, to a DNA template;

elongating the annealed primer or primers by using a DNA polymerase;

ligating the phosphorylated 5'-terminus and the elongated terminus of the primer or primers by means of a DNA ligase to synthesize a circular DNA containing said primer or primers;

denaturing the circular DNA;

repeating the reactions of annealing, elongating, ligating, and denaturing to amplify the circular DNA to generate DNA products including a multiple copies of a single-stranded circular DNA containing the primer or primers;

selectively digesting the DNA products other than the single-stranded circular DNA to produce megaprimer fragments;

annealing said megaprimer fragments to said single-stranded circular DNA;

elongating the annealed megaprimer fragments by using said DNA polymerase to synthesize a double stranded DNA; and

adding an auxiliary primer complementary to a region adjacent to the nucleotide sequence in which mutations are introduced,

wherein said auxiliary primer is a T7 primer.

12. (Currently Amended) A method for mutagenesis comprising:

annealing one or more primers having a nucleotide sequence containing at least one mutation and a phosphorylated 5'-terminus, to a DNA template;

elongating the annealed primer or primers by using a DNA polymerase;

ligating the phosphorylated 5'-terminus and the elongated terminus of the primer or primers by means of a DNA ligase to synthesize a circular DNA containing said primer or primers;

denaturing the circular DNA;

Applicant : Atsushi Miyawaki et al.
Serial No. : 09/920,922
Filed : August 2, 2001
Page : 5 of 6

Attorney's Docket No.: 11283-012001 / PH-1270US

repeating the reactions of annealing, elongating, ligating, and denaturing to amplify the circular DNA to generate DNA products including a multiple copies of a single-stranded circular DNA containing the primer or primers;

selectively digesting the DNA products other than the single-stranded circular DNA to produce megaprimer fragments;

annealing said megaprimer fragments to said single-stranded circular DNA; and

elongating the annealed megaprimer fragments by using said DNA polymerase to synthesize a double stranded DNA,

wherein a thermostable high-fidelity DNA polymerase and/or a thermostable DNA ligase are used in synthesizing the single-stranded circular DNA and the double-stranded circular DNA.